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Sexual selection in a tropical fruit fly: role of a plant derived chemical in mate choice

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Running title: Raghu et al. ! ME feeding and mate choice in a dacine fruit fly

Abstract

The specific mechanisms by which selective pressures affect individual species are often difficult to resolve. In tephritid fruit flies males respond strongly and positively to certain plant derived chemicals. Sexual selection by female choice has been hypothesized as the mechanism driving this behaviour, as females preferentially mate with males that have fed on these chemicals. This hypothesis is, to date, based on studies of only very few species and its generality is largely untested. We tested the hypothesis in small cage, semi-natural (field cage) and natural environments using a monophagous fruit fly, *Bactrocera cacuminata*. This species is known to respond to methyl eugenol (ME), a chemical found in many plant species and one upon which previous studies have focused. We found that males fed on multiple occasions on this chemical, but contrary to expectation no obvious female choice was apparent in selecting ME-fed males over unfed males as measured by the number of matings achieved over time, copulation duration or time of copulation initiation. However, the number of matings achieved by ME-fed males was significantly greater than unfed males 16 and 32 days after exposure to ME in small cages (but not in a field cage). This delayed advantage suggests that ME may not influence the pheromone system of *B. cacuminata* but may have other consequences. Female choice may therefore be passive or cryptic, acting on some other fitness consequence (e.g. enhancement of physiology or survival) of male exposure to these chemicals. We discuss the ecological and evolutionary implications of our findings to explore alternate hypotheses to explain the patterns of response of dacine fruit flies to specific plant-derived chemicals.

Keywords: Female choice – methyl eugenol – parapheromone – feeding – mating – sexual selection – *Bactrocera cacuminata*.

Introduction

Darwin (1871) speculated that forces other than natural selection influence the nature of species, in particular he realized that an equally powerful influence could be sexual selection. He hypothesized that sexual selection could be operating by competition between members of one sex to gain access to conspecific mates (intrasexual selection), or due to the preferences of one sex to attributes of the other with associated fitness consequences (intersexual selection). Since his initial proposal, sexual selection has continued to be a major focus of theoretical and empirical studies (Andersson, 1994; Bateson, 1983; Bradbury and Andersson, 1987; Fisher, 1930; Gross, 1994; Hamilton and Zuk, 1982; Kirkpatrick, 1982; Lloyd, 1979; Maynard Smith, 1985; O' Donald, 1980; Thornhill and Alcock, 1983; Zahavi, 1975).

The underlying mechanisms of sexual selection (i.e. male-male competition and female choice) are often difficult to resolve (Andersson, 1994; Eberhard, 1997; Ryan, 1997; Searcy, 1982) and may not be independent of each other. For example, in certain fish species (Candolin, 1999; Morris et al., 1995) and barn swallows (Galeotti et al., 1997), male-male competition influences female choice. It has been noted that difficulties of resolving the mechanisms of sexual selection are particularly evident in insect species (Conner, 1988; Pormarcom and Boake, 1991; Thornhill and Alcock, 1987).

For one group (Dacinae) within the insect family Tephritidae (true fruit flies; Insecta: Diptera), sexual selection has been hypothesised to occur through a mechanism of female choice (Shelly, 2000). Female flies are believed to preferentially mate with males that have fed on a group of chemicals known to fruit fly biologists as

parapheromones or male-lures. These chemicals occur naturally in plants (e.g. methyl eugenol) or are close analogues of plant derived chemicals (e.g. cue-lure) (Fletcher, 1987; Fletcher et al., 1975; Sivinski and Calkins, 1986). The parapheromones elicit strong anemotaxis in male flies and, at least in some species, equally strong chemotactic feeding responses (Meats and Hartland, 1999; Meats and Osborne, 2000). The ingested substances are hypothesized to be integrated into the male fly's sex pheromone system (Fitt 1981; Nishida et al., 1988, 1993, 1997), subsequently making those males more attractive to females (Shelly, 2000). Female choice is believed to have resulted in a runaway process (sensu Fisher, 1930) that has been provided as the reason for the exceptionally strong response of male fruit flies to these chemicals (Shelly, 2000).

While this hypothesis seems plausible its generality is unclear, as there is considerable variability in the known behaviour of different fruit fly species to different lures. For example, *Ceratitis capitata* does not ingest trimedlure (Shelly and Dewire, 1994; Shelly et al., 1996) and would appear to counter the hypothesis, while yet other fruit fly species have no known male lure response (Drew and Hancock, 1994) and may operate under totally different mechanisms of mate recognition and acceptance. In contrast, *Bactrocera dorsalis*, the best-studied species, strongly supports the hypothesis. While it is thought to feed on methyl eugenol only once, with repeat feeding a rarity (Shelly, 1994), methyl eugenol fed males do have enhanced mating competitiveness over unfed males (Shelly and Dewire, 1994; Tan and Nishida, 1996). Unfortunately, a lack of similar work on other parapheromone responding dacine species makes it difficult to judge if the results from *B. dorsalis* are widespread in dacine flies, or if it is a species specific characteristic.

In this paper we examine the hypothesis that methyl eugenol feeding enhances male mating success through the subsequent production of a “superior” male sex pheromone (the female choice or intersexual selection model). As our study animal we use the dacine fruit fly *Bactrocera cacuminata* (Hering), which occurs in same taxonomic complex as *B. dorsalis* (Drew 1989; Drew and Hancock 1994).

Specifically in this paper we investigate the following questions.

Feeding behaviour:

1. Does feeding occur on ME and how often?
2. Is there a pattern in the frequency of feeding on ME?
3. Is the frequency and duration of feeding on ME related to time spent feeding on ME on a previous occasion?

Mating behaviour:

1. Do females preferentially mate with ME-fed males over males that have not fed on ME?
2. Is the pattern of mating in relation to prior exposure to ME consistent among small cage, field-cage and field experiments?
3. Does ME serve as a precursor to a short-range pheromone or a long-range pheromone?

Methods

Bactrocera cacuminata is a non-pest, monophagous species that utilizes *Solanum mauritianum* Scopoli as its host plant. It is a member of the *dorsalis* complex of fruit flies (Drew, 1989; Drew and Hancock, 1994), which includes *B. dorsalis* on which

previous tests of Fitt's (1981) hypothesis have been done (Shelly, 1994; Shelly and Dewire, 1994; Tan and Nishida, 1996). *Bactrocera cacuminata* males also respond strongly and positively to ME: in one field trial over 23,000 flies were caught in only 10 Steiner traps over a 10 day period (Raghu et al. – in press). This fly therefore seems an appropriate candidate to examine the generality of the methyl eugenol as a pheromone precursor model in dacine ecology.

All flies used in the experiment were from a colony maintained at Griffith University. Flies used in the feeding behaviour and glass house experiments were 8 generations old, while those used in the field cage and field experiments were 16 generations old. Wild flies were released into the colony every 2-3 generations to minimise the effects of any laboratory-induced selection pressures.

Adult flies were separated by sex within two days of emergence, well before they attain sexual maturity at approximately 10-14 days. No more than 100 adult flies were maintained in 30 x 30 x 30cm screen cages with water, sugar and protein provided *ad libitum*. The flies were maintained in a rearing room at a temperature of 25-27°C and 65-70% relative humidity. The rearing room was under semi-natural light conditions, with fluorescent tubes illuminating the room between 0800 and 1600h and natural light for the remainder of the day.

Feeding behaviour

Fifty sexually mature male flies (14 days old) were selected and housed individually in clear plastic containers (18 x 12 x 6cm: length x width x height) under natural light conditions. The flies had access to food (sugar + protein hydrolysate) and water

continuously during the course of the experiment. Each day for 14 days, one ml of methyl eugenol (ME) on a cotton wick (2cm long x 1cm diameter) was provided to each of the flies for a period of thirty minutes between 1100-1200h. This period was selected as it has been shown to be the peak feeding period of *B. cacuminata* on ME (Brieze-Stegman et al., 1978). Continuous observations were made over the 30 minute period to document whether each individual fly was feeding, the number of feeding events/ bouts and duration of each feeding event per fly.

Mating behaviour

Mating in *B. cacuminata* has been studied in considerable detail in the laboratory (Myers, 1952) and is restricted to dusk (Fletcher, 1987).

Male flies used in these studies were separated into a treatment and control group. The former was exposed to 2ml of ME on a cotton wick for a continuous 24 hour period beginning at 0600 hours. Flies were observed to feed on the wick within five minutes of initial exposure to the wick. The age of flies at time of exposure was 14 days and hence they were sexually mature.

Small cage experiments

On the day of exposure (Day 0), 5 ME-fed virgin males, 5 unexposed virgin males (hereafter referred to as unfed) and five virgin females were released in to each of ten clear perspex cages (40 x 40 x 40 cm) at 1500h. Each cage contained a terminal portion of a *S. mauritanum* branch that comprised a cluster of fruit and a whorl of leaves, with the stalk immersed in a flask of water. The cages were housed in an ambient temperature glasshouse under natural light conditions. Prior to the release of

the flies, treatment and control males were cooled ($\approx 10\text{-}12^{\circ}\text{C}$) and marked with a different colour on the thorax. Preliminary analyses indicated that such marking had no effect on mating competitiveness ($\chi^2 = 0.0196$, $df = 1$, $P = 0.8885$). As prolonged cooling has a potential to influence behaviour (Barron, 2000), care was taken not to expose flies to low temperatures for more than ten minutes. Flies were observed to resume normal activity within 5 minutes of being released into the cage.

Continuous observations were made from 1600 to 1930h (full night). Details of any courtship, time of initiation and duration of copulation and status of male in copula (ME-fed vs. unfed) were recorded. If copulation had not terminated by the end of the observation period, observations were made at 0600h the following morning to determine if flies remain coupled during the night or terminated copulation during the night. If copulation had terminated during the night the duration of copulation was calculated to be the time between end of the observation period (1930h) and the time of copulation initiation. This method consistently underestimated the duration of copulation. The trial was repeated on days 1, 2, 4, 8, 16 and 32 after exposure to ME. These day intervals were chosen as they are similar to previous experiments (Shelly and Dewire, 1994).

Field-cage experiments

A cylindrical field cage (230cm high \square 250cm diameter) was set up housing three potted *S. mauritanum* plants. At similar intervals to the small cage experiments, 10 ME-fed, 10 unfed and 10 virgin females were released into the field cage.

Observations were made from 1600 to 1930h and data similar to that in the small cage experiment were gathered.

Field experiments

If ME serves as a precursor for a long-range pheromone then the previous experiments in confined environments may not detect its influence. We therefore ran field experiments to help investigate this.

In the field individual flies or groups of five flies, again either ME-fed and unfed, were enclosed in clear cylindrical tubes (9cm long \square 3cm diameter). The tube ends were sealed with gauze to permit airflow. These tubes were suspended from each of five *S. mauritianum* host plants or five neighboring (\approx 5 metres distant) non-hosts, *Lantana camara*. The host plants had wild flies present on them and were selected from a continuous patch along a creek in Brisbane (27° 28' S, 153° 2' E). A control tube containing no flies was also suspended at each plant. Thus on each of 10 plants (five host, five non-host) was hung a tube with single flies of each status (ME-fed and unfed), a tube with 5 flies of each status (ME-fed and unfed) and a control tube (with no flies). These tubes were hung on plants at 1500h on days 0, 1, 2, 4, 8, 16 and, 32 after exposure to ME. Each tube was coated with Tanglefoot[®] (The Tanglefoot Company, Grand Rapids, Michigan, U.S.A.) to trap wild flies attracted to the caged males. Additionally, continuous observations were made from 1600 to 1930 h to visually record whether wild flies were attracted to the tubes and/or adjacent foliage.

Flies in all mating behaviour experiments were only used once.

Data Analysis

Data from the feeding behaviour experiments were analyzed using a χ^2 Goodness of fit test. The frequency distribution of the number of times a fly fed on ME over the entire experiments and the duration since last feeding were tested against a random Poisson distribution (Zar, 1999), as feeding is expected to be a rare event. In addition the relation between the duration of feeding event (seconds) and the time (in days) till subsequent feeding and duration of subsequent feeding (seconds) were examined using non-parametric correlation analyses.

A logistic regression analysis to test the effect of exposure to ME on mating success of male *B. cacuminata* (Zar, 1999). The effect of exposure to ME on time of copulation initiation and copulation duration (time mating pair remained coupled) was investigated using the Kruskal–Wallis test and univariate analysis of variance respectively, with status (ME-fed vs. Unfed) as the factor. Data for these analyses were pooled across days for all mating behaviours as there were no significant within day differences, either in time of copulation initiation or copulation duration, between flies of either status.

The effects of exposure to ME on mating success of males in the field cage study was analyzed using binomial tests (Conover, 1999), to test if the ratio of ME-fed males to unfed male differed significantly from 1:1, on each of the days observations were made.

No wild *B. cacuminata* were attracted to our field experiments and hence no analyses were possible. We address why this could have happened in our Discussion.

Results

Feeding behaviour

Four flies died during the course of the experiment and any data pertaining to them have been excluded from analyses. Flies were observed to feed on methyl eugenol on all days of the experiment (Figure 1a). The frequency distribution of the number of times a fly fed over the entire experiment was not significantly different to a random Poisson process ($\chi^2 = 7.827$, $df = 5$, $p = 0.1634$; Figure 1b). Only two flies did not feed at all throughout the experiment (Figure 1b). Most flies fed multiple times with the modal frequency of three times over the entire experiment and a median frequency of three. The frequency distribution of the duration since last feeding event was significantly different from a random Poisson process ($\chi^2 = 82.1722$, $df = 5$, $p < 0.0001$; Figure 1c). Where flies fed on multiple occasions, the modal duration between feeding events was one day with a median duration of two days. Multiple bouts of feeding within a day were observed per fly (mean \pm standard error = 2.02 ± 0.07 bouts/ fly/ day), with one fly feeding on 8 separate occasions within a single day. Duration of individual feeding bouts varied considerably (mean \pm standard error = 260.63 ± 15.06 seconds/ bout; range 30-1800 seconds/ bout). The mean duration of feeding was variable (Figure 1a) with the longest average duration on days one and four. Flies fed for a considerable duration on each of the days they were exposed to ME (Figure 1a).

Spearman's rank-order correlation coefficient revealed that there was no significant relationship between duration of feeding event (time of all bouts combined for a day) and time in days till next feeding event ($r_s = 0.049$, $n = 110$, $p = 0.611$; Figure 2a). There was no relationship between duration of feeding by *B. cacuminata*

and duration of the subsequent feeding event ($r_s = 0.074$, $n = 110$, $p = 0.440$; Figure 2b).

Mating behaviour

Small cage experiments

Over the entire small cage experiment more ME-fed males (72 copulations) mated in comparison to unfed males (44 copulations). Logistic regression analyses revealed that status had a significant influence on mating success ($\chi^2 = 6.64$, $df = 1$, $p = 0.01$). However, there was no consistent advantage of males of either status (ME-fed vs. unfed) over time as revealed by the significant interaction effect between status and days since exposure ($\chi^2 = 21.46$, $df = 6$, $p = 0.0015$; Figure 3). Examination of within day variations in mating success between males of different states using univariate analyses of variance, revealed that there was no difference in the number of matings achieved by ME-fed males and unfed males on days 0, 1, 2, 4, and 8 (Figure 3; Day 0 – $F_{1,18} = 0$, $p = 1$; Day 1 – $F_{1,18} = 0.559$, $p = 0.464$; Day 2 – $F_{1,18} = 1.670$, $p = 0.213$; Day 4 – $F_{1,18} = 0.679$, $p = 0.421$; Day 8 – $F_{1,18} = 0.947$, $p = 0.343$). However there was a significant difference on days 16 and 32 with ME-fed males having a much higher mating success in comparison to unfed males (Figure 3; Day 16 – $F_{1,18} = 6.698$, $p = 0.019$; Day 32 – $F_{1,18} = 57.800$, $p < 0.001$).

There was no difference in time of copulation initiation between ME-fed males and unfed males (Kruskal–Wallis $H = 0.162$, $df = 1$, $p = 0.687$; Figure 4a). The duration of copulation also did not significantly differ between unfed males and ME-fed males ($F_{1,114} = 0.978$, $p = 0.325$; Figure 4b).

Field-cage experiments

Binomial tests comparing the proportions of copulations achieved by ME-fed males and unfed males revealed that there was no significant difference between males of either state (Figure 5).

Field experiments

Despite carrying out all replicates of the trial on all days, no wild flies were trapped on the tubes, nor were wild flies seen on adjacent foliage.

Discussion

The use of lures and attractants in the control of insects is quite common (Howse et al., 1998). In some cases the precise biological/ ecological reason underpinning these attractants are well understood. However, for certain insects, such attractants have been fortuitously discovered and the biological basis for their success remains an enigma (Carde and Minks, 1997; Hardie and Minks, 1999). Dacine fruit flies are one such group of insects. In spite of the widespread use of lures in fruit fly management, questions on their role in the ecology and evolution of fruit flies remain largely unresolved, although Shelly (2000) proposed that sexual selection, specifically female choice, was influential in male response to lures. Hence we investigated the feeding behaviour on one such lure (methyl eugenol) and associated mating consequences in a monophagous, non-pest dacine fruit fly.

Our results show that, in small container situations, multiple feeding on ME is a common occurrence in *B. cacuminata* (Figure 1b), with many individuals feeding on

successive days (Figure 1c). One explanation for this could be that repeat feeding was only occurring in flies that were consuming small amounts of ME during first feeding. Assuming that feeding duration is a reliable indicator of ME intake, the poor correlations between duration of feeding and time to next feeding (Figure 2a) and duration of subsequent feeding (Figure 2b) suggests that this is an unlikely explanation. If ME is a precursor to a pheromone that sexually mature males release at dusk, then repeat feeding should not be unexpected. Pheromones are highly volatile and multiple feeding on a precursor chemical is likely to be common so as to allow males to replenish pheromones for subsequent release.

Investigation of the influence of ME on mating success revealed that ME-fed males in the small cage were more successful in mating than unfed males only on Day 16 and Day 32 after exposure to ME (Figure 3). If females were preferentially choosing ME-fed males, these males may be expected to have an advantage in terms of copulation duration, with the selected males prolonging insemination or remaining in postcopulatory/ postinsemination contact as a form of mate guarding (Alcock, 1994; Andersson, 1994; Field et al., 1999; Radwan and Siva-Jothy, 1996; Yuval et al., 1990). Similar benefits may be expected in terms of time of copulation initiation (i.e. advantaged males might begin copulation earlier). However, our results indicate that there is no difference between ME-fed and unfed males in copulation duration or time of copulation initiation (Figure 4). These results are contrary to those of Shelly and Dewire (1994) and Tan and Nishida (1996).

Why do ME-fed males have a mating advantage on days 16 and 32, but not on days prior to that? Could this be the physiological processing time for the

transformation of ME (the precursor) into the pheromone? This appears unlikely given that transformation of precursors into pheromones is quite rapid in insects (Tillman et al. 1999; C. J. Moore – personal communication). In the case of fruit flies, Nishida et al. (1988) reported that the transformation of ME into metabolites identified from the rectal gland took between 1-3 days. Hence, it is evident that if methyl eugenol is a precursor to the male sex pheromone, ME-fed males should experience higher mating success much earlier than observed in the present study. The absence of mating advantage in the field cage experiment and the non-response to ME-fed individuals and clusters in the field experiment further suggests that ME (as a precursor to a long distance pheromone) may not be critical in the mating system of *B. cacuminata*. Given these findings and the results of the present study, there does not appear to be a simple explanation, using Fisher's runaway sexual selection model via female choice, to clarify the basis for the strong attractance of male *Bactrocera cacuminata* to methyl eugenol.

This study indicates no obvious female choice for ME-fed males over unfed males. An alternative explanation could be that feeding on ME confers some other physiological benefits to males, such as enhanced energetic reserves and this is the basis for female choice (referred to as metabolic competence by Watson and Lighton 1994). This is not uncommon in insects that feed on phytochemicals believed to be pheromone precursors (Arnold and Houck, 1982; Field and Yuval, 1999; Thornhill and Alcock, 1983; Tillman et al. 1999; Yuval et al., 1998). However, our recent study exploring the physiological significance of ME to *B. cacuminata* (Raghu et al. – in press) indicates no such enhancement of metabolic competence.

Exploring alternate hypotheses

If there are no reproductive or metabolic benefits of feeding on methyl eugenol by *B. cacuminata*, then how does one explain this strong chemotactic response of the species? Could it serve a function in larval host plant location? Though many dacine species respond to methyl eugenol, this phenyl propanoid is not commonly found in larval host plants of fruit flies. However, it does occur in numerous plant species, including some orchids (Nishida et al. 1993). This phenomenon of chemotaxy towards a chemical not currently associated with host plants is not unique to dacine fruit flies. Cucurbitacins (terpenes produced by all members of the Cucurbitaceae) elicit strong phagostimulatory response in many luperine (Chrysomelidae: Luperini) beetles that develop only on non-cucurbitaceous host plants (Metcalf et al. 1980). This response has been hypothesised to be a relic of ancestral host associations of luperine beetles with members of the Cucurbitaceae that is currently being maintained through secondary selection for contemporary benefits of cucurbitacin feeding, such as defense (Ferguson and Metcalf 1985, Tallamy et al. 1998). This has been labelled the “ancestral host hypothesis” by Tallamy et al. (1999).

Defensive benefits as a result of feeding on methyl eugenol have been hypothesized for male dacine fruit flies. The Asian house gecko (*Hemidactylus frenatus* Duméril and Bibron) is deterred from feeding on methyl eugenol fed *Bactrocera papayae* Drew and Hanacock males and culex fed *B. cucurbitae* (Coquillett) males (Tan and Nishida 1998, Tan 2000). If methyl eugenol confers similar allomonal benefits to *B. cacuminata* then feeding on this phytochemical can still be regarded as pharmacophagy. Though we did not quantify predation, our recent studies (Raghu et al. – in press [not in reference list]) exploring this issue suggest that

feeding on methyl eugenol did not enhance survival in the presence of potential predators in the field cage.

Recently, Tallamy et al. (1999) provided an alternative to the ancestral host hypothesis (see Metcalf [1990] for discussion of the ancestral host hypothesis with respect to the Dacinae) that may explain the strong phagostimulatory responses elicited in dacine fruit flies by phytochemical lures. Central to their idea is the ease with which “loose” gustatory receptors of insects associate with novel plant compounds, given that a wide variety of molecules meet the polarity and configuration requirements of these receptors. However the “loose receptor hypothesis” requires that exposure to the phytochemical must be frequent enough to select for physiological tolerance and provide distinct reproductive and/ or defense benefits to the consumer for selection to favor such a response (Tallamy et al. 1999).

The applicability of the “loose receptor hypothesis” (Tallamy et al. 1999) to dacine response to parapheromones is unclear. The availability of methyl eugenol in an accessible form in nature is unclear, despite the fact that it has been recorded as a phytochemical component of a variety of plants (Shelly and Dewire 1994, Shelly 2000). Furthermore the feeding behavior and associated reproductive benefits of feeding on these phytochemicals are not consistent between species (Shelly and Dewire 1994, Tan and Nishida 1996, Shelly 2000, Raghu et al. – in review [in press? Or something else??]). While defensive benefits suggested in the two dacine species are insightful (Tan and Nishida 1998, Tan 2000), such benefits need to be determined for predators in natural systems and habitats in which these species evolved. Only

then can we understand the selection pressures that could have resulted in such a strong phagostimulatory and/ or orientation response.

The biological basis of attraction to ME remains an enigma. Biosynthetic pathways of phenyl propanoids (such as methyl eugenol and cuelure) of relevance to fruit flies are being investigated by organic chemists (Fletcher and Kitching 1995). However to place this information in an evolutionary framework, it is vital to understand the phylogenetic relationships between the dacine fruit flies in relation to lure response. This needs to then be examined in the context of phylogenetic relationships of the host plants of the Dacinae and associated phytochemistry. Only such a collaborative effort between entomologists, botanists and biochemists can unravel ecological and evolutionary significance of phytochemical lures to dacine fruit flies.

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Figure legends

Fig. 1. Feeding behaviour of male *Bactrocera cacuminata* on methyl eugenol. (a) Number of individuals feeding on methyl eugenol on each day of exposure (bar) and mean duration of feeding \square standard error (filled circle). (b) Frequency distribution of number of times an individual fly fed over the entire experiment. (c) Frequency distribution of interval between successive feeding events.

Fig. 2. The duration of feeding on methyl eugenol (in seconds) by male *Bactrocera cacuminata* correlated with (a) Time (days) till subsequent feeding event and (b) Duration of subsequent feeding event (in seconds).

Fig. 3. The relative mating success of methyl eugenol fed *Bactrocera cacuminata* males versus unfed males over time in small cage experiments. Shaded bars represent methyl eugenol fed males and open bars represent unfed males. The letters above the bars represent outcomes of univariate analyses of variance. Same letters on adjacent bars on any given day indicate no significant difference ($p > 0.05$) in mating success between ME-fed and unfed males.

Fig. 4. Effect of exposure to methyl eugenol on copulation. (a) Time of copulation initiation (hours) in relation to status (methyl eugenol fed or unfed) of copulating male. (b) Copulation duration (seconds) in relation to status (ME-fed or unfed) of copulating male. $n = 72$ for methyl eugenol fed males; $n = 44$ for unfed males for both graphs.

Fig. 5.

The relative mating success of methyl eugenol fed males versus unfed males over time in a large field cage. Shaded bars represent methyl eugenol fed males and open bars represent unfed males. The letters above the bars represent outcomes of binomial tests. Same letters on adjacent bars on any given day indicate no significant difference ($p > 0.05$) in mating success between ME-fed and unfed males.

Figure 1
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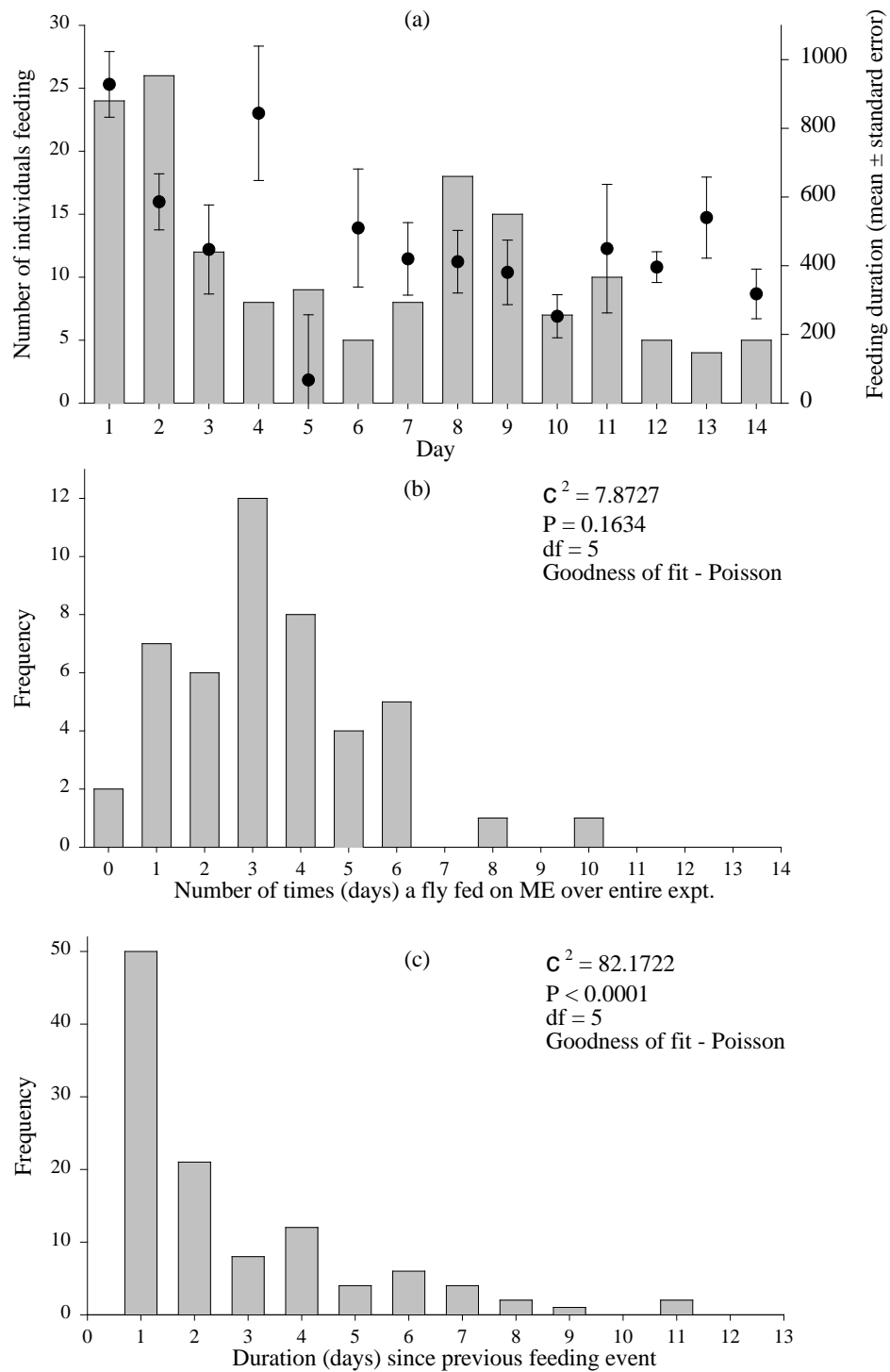


Figure 2
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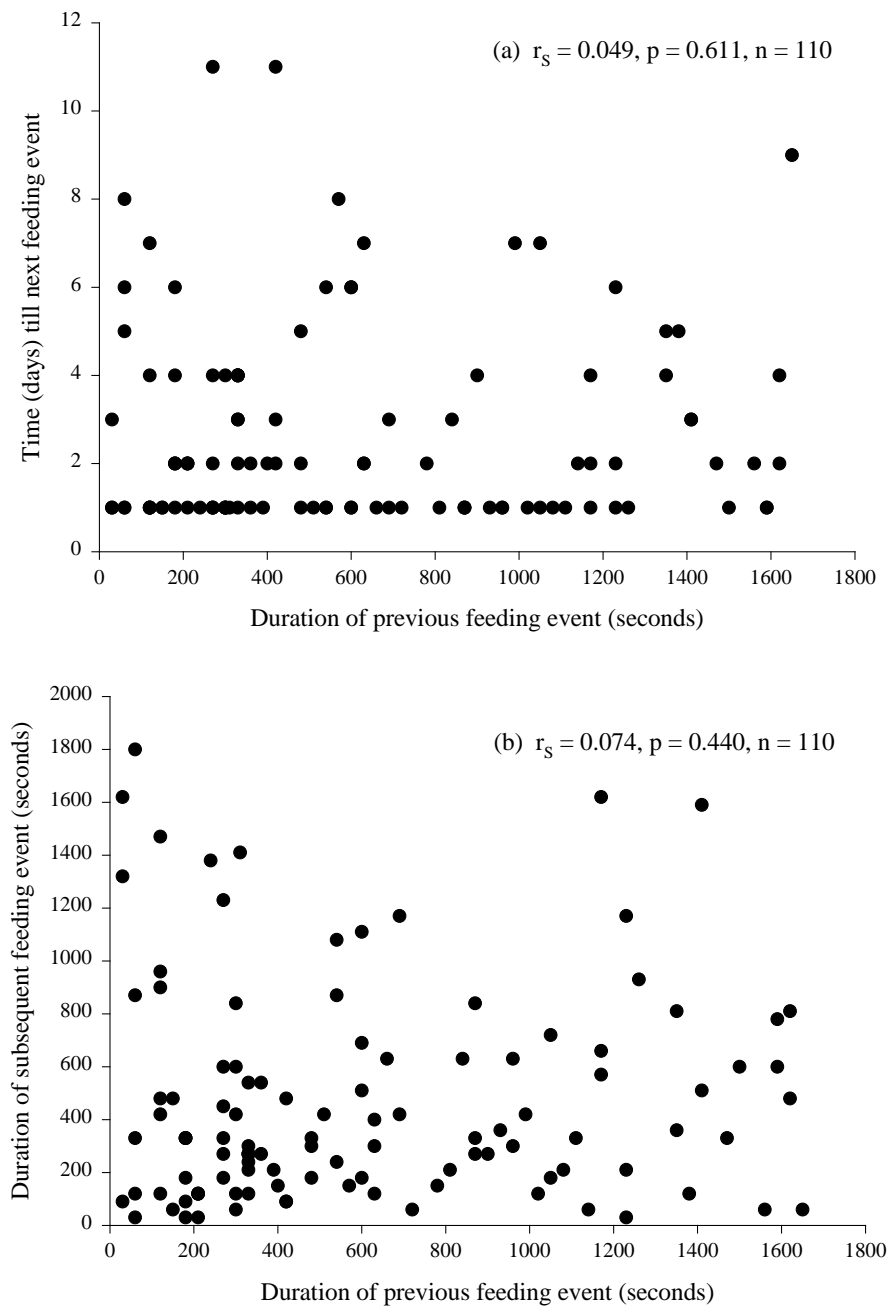


Figure 3
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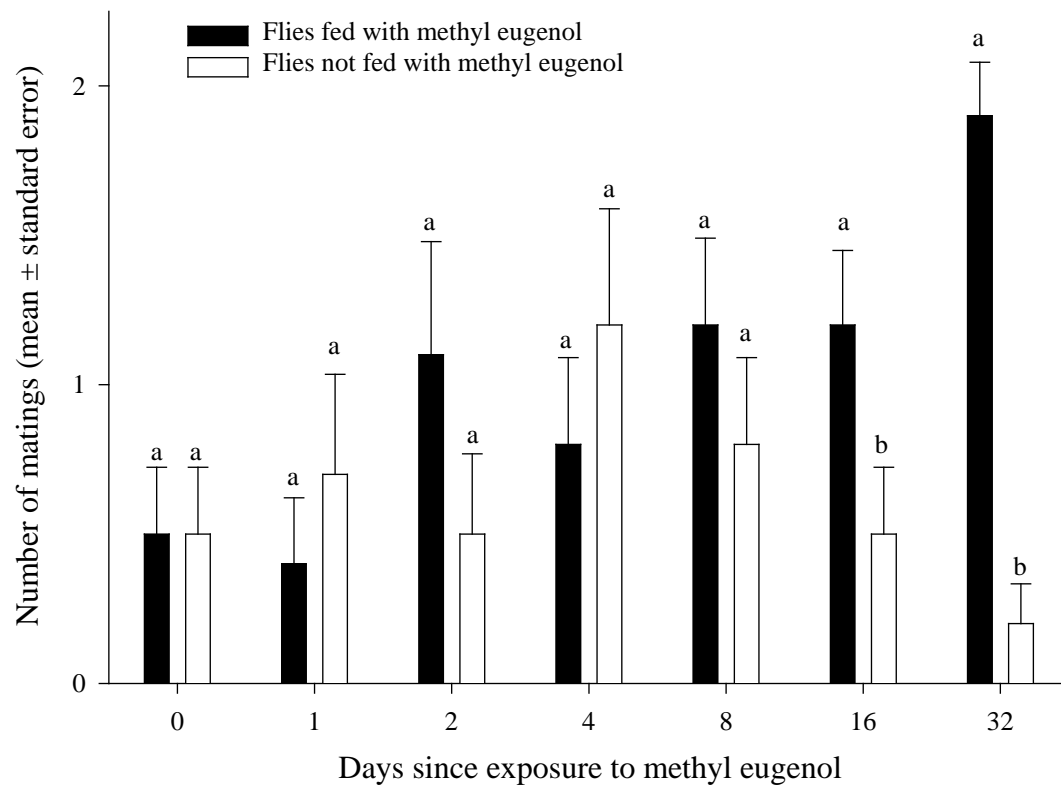


Figure 4
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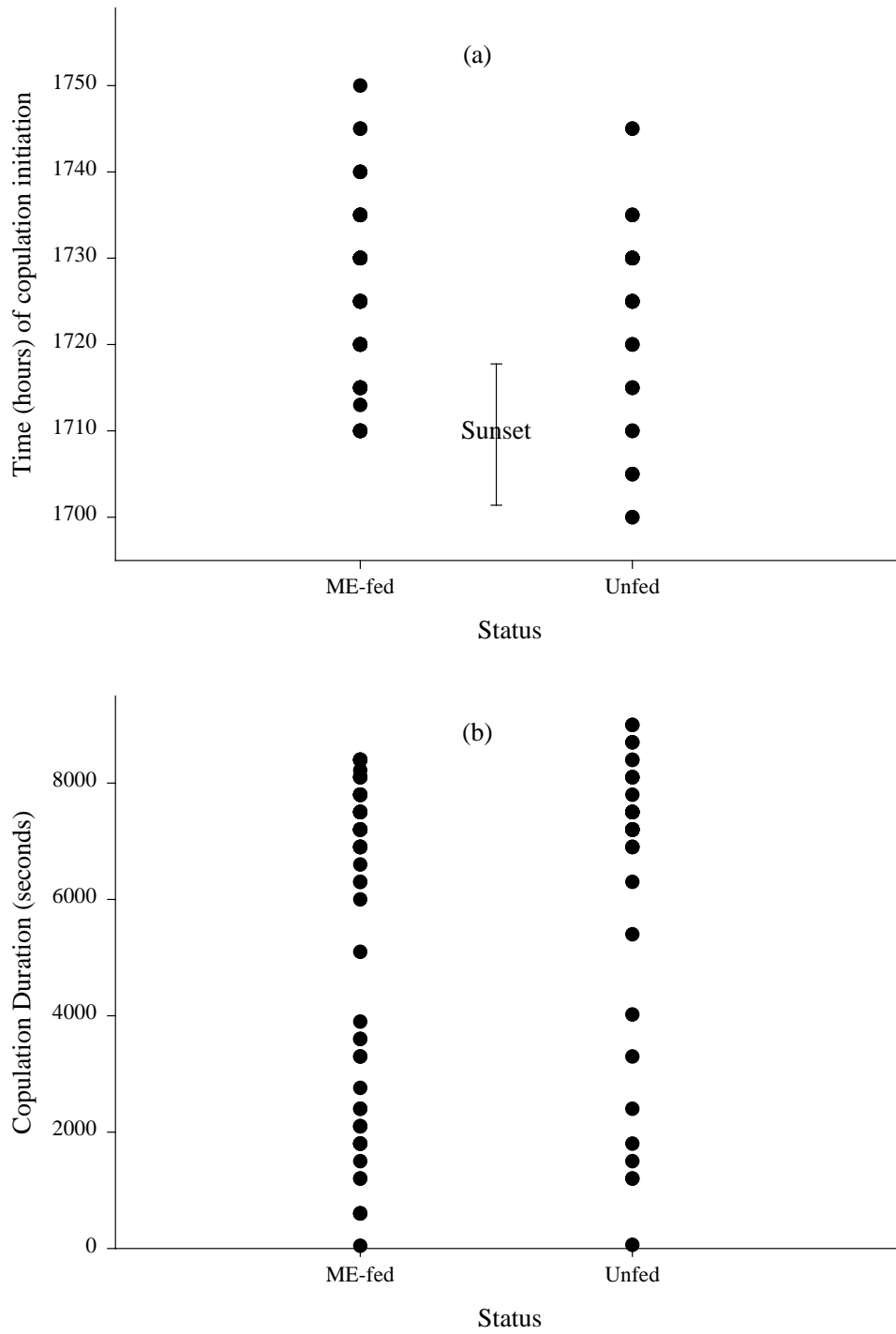


Figure 5
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